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"Solidarity in a competing world — fair use of resources"

Nitrate Reductase Activity as Potential Indicator for Biological Nitrification Inhibition in *Brachiaria humidicola*

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Abstract

"Biological Nitrification Inhibition" (BNI) is a naturally occurring process by which soil nitrogen (N) is conserved in the less mobile form of ammonium (NH_4^+) resulting in a reduction of nitrate (NO_3) leaching losses and N_2O emissions. The pasture grass *Brachiaria humidicola* (Bh) is currently the most prominent species showing potential to naturally inhibit nitrification. This research aimed to identify *in situ* plant physiological indicators for BNI activity and to evaluate Bh accessions for their BNI potential with a rapid and reliable methodological approach under field conditions, proposing a direct linkage between nitrification deriving NO_3 in soil and nitrate reductase activity (NRA) in plants. *In vivo* NRA in leaf was determined after N fertilisation and synchronised with soil sampling to test the relationship between soil nitrification rates and NRA. Soil N-NO_3 was determined *in situ* to facilitate correlation analysis. The genotypes tested included two *B. humidicola* CIAT germplasm accessions (CIAT 16888 and CIAT 26146) as controls and four *B. humidicola* hybrids that were preselected based on greenhouse evaluation of BNI activity in soil. Beforehand enzyme substrate (NO_3) inducibility was clearly verified and plant leaves turned out to be the main tissue of NO_3 reduction (roots vs. leaves). The high BNI Bh accession (CIAT 16888) showed the lowest NRA, whereas the low BNI accession (CIAT 26146) showed highest NRA among all the tested materials. Previously, Bh-08–679 and Bh-08–675 were identified as high BNI and low BNI hybrids, respectively. The NRA assay confirmed these previous observations. Two Bh hybrids (Bh-08–700 and Bh-08–1149) showed an intermediate level of BNI potential. Methodological comparison (soil nitrification rates vs. *in vivo* NRA) resulted in discrepancies concerning ranking of BNI capacity. NRA in plant tissue correlated well with soil N-NO_3 concentration (R^2=0.81, $p < 0.05$). This significant positive correlation between NRA vs. N-NO_3 concentration in the soil indicates that plant tissue NRA can be a potential indicator of BNI in soil. Further research is needed to estimate to what extent the high BNI Bh hybrids can contribute to N conservation in soil to benefit resource-poor smallholders in the tropics where synthetic nitrification inhibitors are expensive and less effective.

Keywords: Forages, nitrogen cycle, tropical grasslands

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